Vol.57, n.1: pp. 45-47, January-February 2014
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

The Constitutive Expression of the V Gene of *Parainfluenza virus* 5 Affects the Growth Properties of Bovine Herpesvirus 5

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ABSTRACT

This study aimed to analyze the effect of the expression of Parainfluenza *virus 5 (PIV5) V protein in bovine cells on the replication of Bovine herpesvirus 5 (BoHV-5). Growth properties of BoHV-5 were evaluated in parental and PIV5 transfected cells. In one-step growth experiments, the BoHV-5 reached higher titers at earlier time points in the transfected cells when compared to the parental cells. The mean plaque size produced by the BoHV-5 in transfected cells was larger than the parental cells. This indicated that the expression of the PIV5 V gene facilitated the release and cell-to-cell spread of BoHV-5 in bovine cells.*

Key words: BoHV-5, V mRNA, transfection

INTRODUCTION

Bovine herpesvirus 5 (BoHV-5), the etiological agent of neurological infections of cattle (Delhon et al. 2003), is able to induce different mechanisms of innate and acquired immunity. An important outcome of innate antiviral responses is the production and secretion of type I interferons (IFN-I, e.g. IFNα and IFNβ) and subsequent auto and paracrine activation of signaling pathways via IFN-I receptors, which ultimately limit virus replication and spread. Following virus infection, the induction of IFN-I occurs in three phases and requires activation of different signaling pathways. In the first phase, pattern recognition receptors (PRRs, e.g Toll-like receptors) interact with the conserved viral motifs, resulting in the production of IFNβ. In the second phase, the binding of IFNβ to its IFN-I receptor and signaling via the Signal Transducer and Activator of Transcription molecules STAT1 and STAT2 activates the transcription of IFN-stimulated genes (Platanias 2005).

A closely related virus, bovine herpesvirus 1 (BoHV-1), expresses a protein, named bovine infected cell protein 0 (bICP0), which blocks the IFN-I signaling (Everett 2000; Saira et al. 2007). BoHV-5 expresses a bICP0 homolog, and it has

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been recently demonstrated that bICP0 of BoHV-5 can complement its BoHV-1 homolog (Steiner et al. 2010). It is, therefore, likely that the BoHV-5 bICP0 protein is able to block the host IFN-I production in the same way as its BoHV-1 counterpart. The present study analyzed the interactions between the IFN-I receptor signaling and the replication of BoHV-5.

MATERIALS AND METHODS

Cells resistant to bovine viral diarrhea virus (CRIB) were transfected with the *Parainfluenza virus* 5 (PIV5) V gene, previously used to block the IFN-I receptor signaling in the bovine cells and subsequently infected with BoHV-5 strain ISO 45/97. The plasmid used for the transfection was pEF.IRES.neo.SV5 V/P, a construct that contained the PIV5 V/P gene as well as the gene for G418 resistance. CRIB cells were transfected with Lipofectamine2000™ (Invitrogen) according to the manufacturer´s instructions. To isolate the cells containing the plasmid, transfected cells were grown in the presence of Geneticin™ (Invitrogen), and resistant colonies were isolated. The Geneticin resistant colonies obtained were submitted to DNA extraction and conventional PCR to detect the V gene. A q RT-PCR using SYBR Green fluorescence was developed in order to detect the presence of the V mRNA. Briefly, total RNA was extracted from the confluent cell monolayers as well as from the non-transfected CRIB cells using RNAqueous Kit (Ambion, Austin, TX) and treated with Ambion® TURBO™ DNase. The RNA was reverse transcribed using Oligo (dT) primers for the cDNAs synthesis. The primers used were based on the sequence of the *Porcine parainfluenza virus* P gene. The positive colonies were named CRIB/V and used for *in vitro* growth experiments. The plaque size assay and one-step growth curves were performed as described previously in triplicate (Franco et al. 2007). Statistical analysis was performed using the Student's *t*-test and the analysis of variance (ANOVA); the least significant difference at $p = 0.05$ was determined. The term "significant" (statistically significant) in the text meant $p \leq 0.05$.

RESULTS AND DISCUSSION

It was estimated that about 10^4 plasmid copies were present in 50 ng of genomic DNA. This low copy number in the CRIB/V colonies could be the result of a natural selection for the cells with a low PIV5 V expression, because overexpression of the PIV5 V protein in the cells could slow down the cell cycle (Lin and Lamb 2000). The qRT PCR revealed the presence of V mRNA in all CRIB/V clones analyzed, indicating that the V protein expression occurred in these cells (data not shown).

The plaque sizes of BoHV-5 in CRIB/V were significantly larger (3.0 +/- 0.4 mm) than the ones in CRIB cells (1.5 +/- 0.8 mm, p= 0.0001, Fig. 1). In agreement with other results, Barreca and O'Hare (2004), after the transfection with the same plasmid, showed that *Human Herpesvirus* 1 (HHV-1) progressed more rapidly and produced larger plaques in MDBK/V cells than in MDBK cells. These results showed that the blockage of the IFN-I receptor signaling had a positive effect on BoHV-5 replication and viral plaque production.

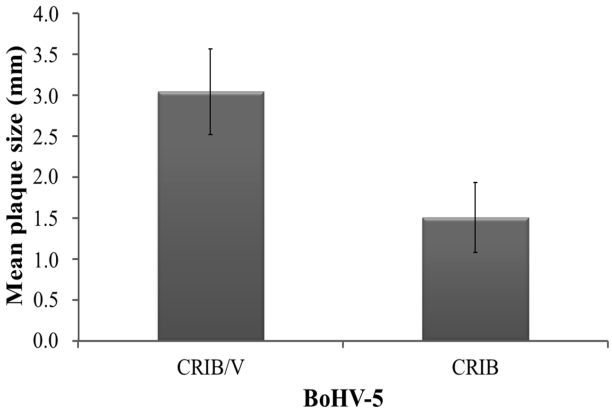

Figure 1 - Graphical presentation of the mean plaque sizes (mm) in CRIB and CRIB/V cells with their standard deviations. The values were estimated by measuring 50 viral plaques of each cell line infected with ISO 45/97 of BoHV-5.

The one-step growth curves of BoHV-5 on CRIB and CRIB/V cells are shown in Figure 2. Infectious viruses were detected earlier in the CRIB/V cells (at 5 h post-infection - p.i) than in CRIB cells (at 9 h p.i.). From 5 h p.i. on until 13 h p.i. the ISO 45/97 titers were significantly higher in the CRIB/V than in the CRIB cells. The maximum difference in virus titers calculated was 1 $\log_{10}$ and occurred at 7 h p.i. From 15 h p.i. on the virus titers in both cells did not differ significantly and the virus reached similar amounts

at 48 h p.i. In previous studies, Young et al. (2003) produced different human cell lines expressing the PIV5 V protein to block the IFN-I receptor signaling and observed that respiratory syncytial virus (RSV) and bunyamwera virus, an *Orthobunyavirus*, could grow to titers 10 to 4000 fold higher in the IFN-I non-responsive cells than in the parental cells. On the other hand, Sherwood et al. (2007) analyzed the replication of human enteric adenovirus type 40 (HAdV-40) in 293-PIV5 V cells in comparison with the parental 293 cells and showed that the resulting virus yields in both cells was more, or less the same at 12 h p.i. It was recently demonstrated that a protein of BoHV-1 (bICP0) blocked predominantly the first phase of the IFN-I induction (Saira et al. 2007 and 2009). Although BoHV-5 bICP0 could act in a similar way, present data indicated that the constitutive expression of the PIV5 V gene and consequent blocking of the IFN-I signaling in CRIB/V was responsible for the observed growth advantage of BoHV-5 in CRIB/V cells, especially when the growth curves (during the first hours post infection) and viral plaque sizes were analyzed.

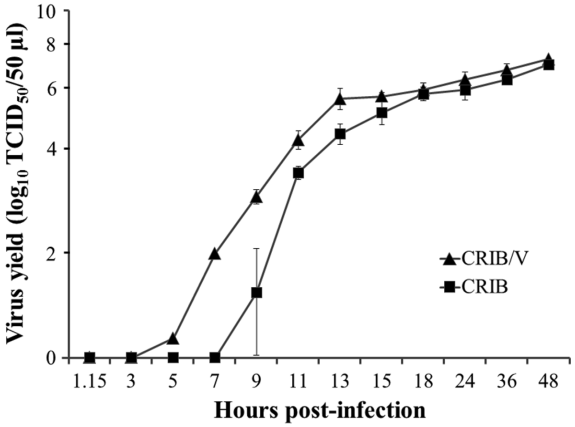

Figure 2 - Growth kinetics of BoHV-5 ISO 45/97 in CRIB and CRIB/V cells. Virus titers are expressed as $TCID_{50}$/50 µL and indicated on a $\log_{10}$ scale.

These results indicated that blocking the signaling via the IFN-I receptor by the PIV5 V protein facilitated the penetration, release and cell-to-cell spread of BoHV-5 in bovine cells. These data suggested that although BoHV-5 blocked (part of) the innate IFN-I responses, it was still sensitive to the antiviral effects of IFN-I during the early stages of infection.

ACKNOWLEDGEMENTS

The authors are thankful to R. Randall for kindly providing the plasmid. Financial support: National Council of Technological and Scientific Development (CNPq). We dedicate this article in memory of Fransciscus Antonius Maria Rijsewijk.

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Received: July 24, 2012;
Accepted: July 02, 2013.